Surface Sampling of *Bacillus* spores

NIOSH DREAM Workshop

Surface Sampling/Biomonitoring Breakout Session

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Acknowledgments

- **Kenneth D. Cole, PhD, Sandra Da Silva, PhD**
  Bioassay Methods Group
  Biochemical Science Division
- **Andy Persily, PhD**
  Indoor Air Quality and Ventilation Group
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- **Steven Choquette, PhD**
  BioaAssay Methods Group Leader
  Biochemical Science Division
- **Laurie E. Locascio, PhD**
  Chief, Biochemical Science Division
- **Bert Coursey, PhD**
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Efforts in Standards for Biological Countermeasures

Standards

Detection

Testing/ Sampling

Guidance

Training

Reference Materials

Training Materials

ASTM E2458 /AOAC

Standard Sampling Methods

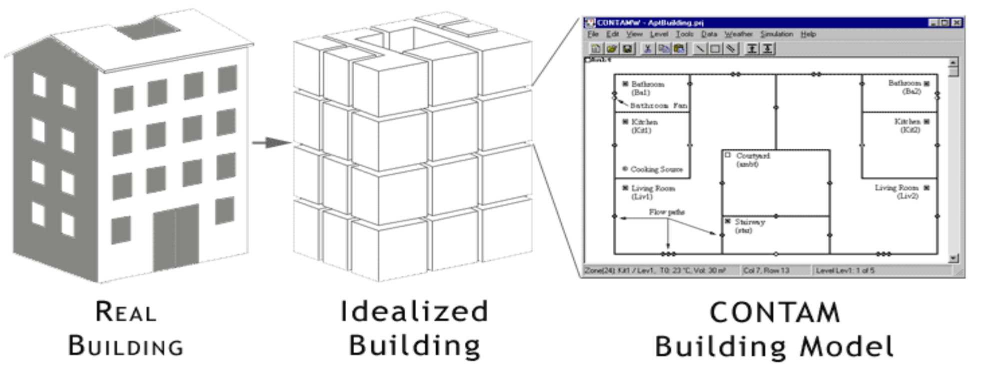
Decontamination

Surface Sampling Validation

CDC and EPA

Sampling Procedures for First Responders

ASTM E2458/AOAC Standard effort
Led by Laurie Locascio



CONTAM/VSP Modeling Software:
Andy Persily at BFRL,
Greg Piepel and Brent Pulsipher at PNNL

- Produce Guidance for the Sampling of Nonvisible Surface Contamination
- Develop a strategy for determining the extent of contamination and decontamination effectiveness

Sampling Visible Powders

The Goal**:** To develop the first US National Standard for collecting suspected biological agents.
The Motivation**:** Motivated by 2005 GAO report calling for validated methods for collecting suspected biological agents.
The Purpose**:** Reduce exposure risk, minimize on site sample consumption, reduce variability (sample handling and analysis).
The Scope: Collection of visible powders, credible biological threat for all biological agents from solid, nonporous surfaces, dispersed in a limited area

ASTM E2458 /AOAC Standard

Includes

- Response Coordination
- Field Screening
- Bulk Powder Sample Collection
- Transportation and Packaging

Bulk Powder Collection

Sterile plastic laminated card used with swab in “broom and dustpan” collection approach

Produces at least 2 samples that are transported to LRN (dry swab in tube, bulk powder in tube)

VSP Work Group

Validated Sampling Plan Work Group is a **DHS led multi-agency work group** set up to address the GAO request.

In a 2005 report titled **Anthrax Detection: Agencies Need to Validate Sampling Activities in Order to Increase Confidence in Negative Results (GAO-05-251),** the Government Accountability Office (GAO) recommended that “to improve the overall process for detecting Anthrax and to increase confidence in negative test results…[DHS] ensure that the overall process of sampling activities, including methods, is validated so that performance characteristics, including limitations, are clearly understood and results can be interpreted.”

The whole sampling process is currently under validation by the CDC, EPA, FBI, NIST, DOD and other agencies.

Current Sampling Methods: Wipe and Swab Procedures

- Deposition method: aerosolized[a,b] (95% Ethanol)
- Wetting agents: Water[a,b] (PBS)
- Controlled Substrata: stainless steel[a], painted wallboard[a]
- Wipes: rayon/polyester blend[a], rayon[b]
- Extraction from Wipe: sonication[a], vortexing[b]
- Report +/- Growth[b], reference coupons[a,b]

Wipe Efficiency Ranges from 7 to 87%

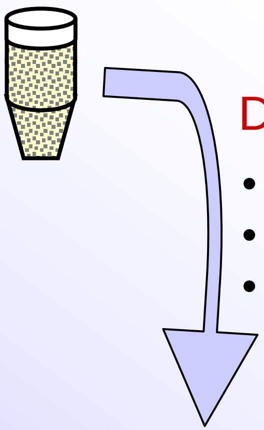
[a]Brown, G. S., R. G. Betty, et al. (2007). "Evaluation of a Wipe Surface Sample Method for Collection of Bacillus Spores from Nonporous Surfaces." Appl. Environ. Microbiol. 73(3): 706-710.

[b]Sanderson, W. T., M. J. Hein, et al. (2002). "Surface Sampling Methods for Bacillus anthracis Spore Contamination." Emerging Infectious Diseases 8(10): 1145-1151.

Challenges in Surface Sampling Methods

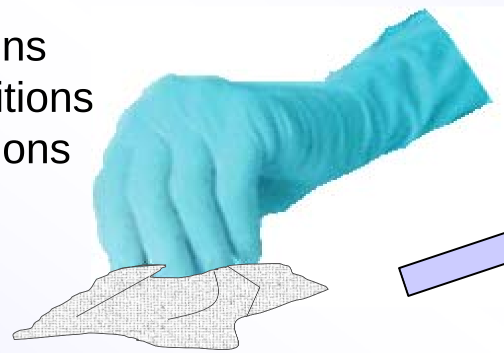
Spore Materials

- Reaerosolization
- Suspension stability
- Viability
- Quantity



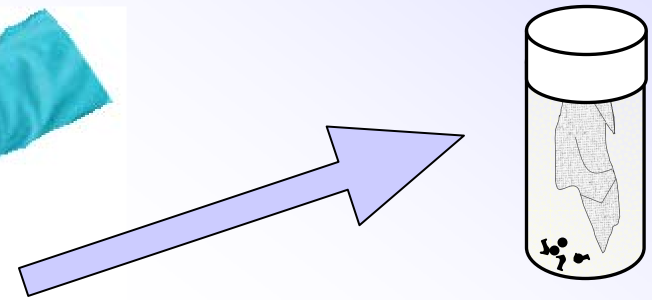
Optimization of Deposition Method

- Solution conditions
- Deposition conditions
- Material interactions



Integration with Detection Technology

- Optimization of removal from wipe
- Interference with detection technologies
- Post-decon impacts on wipe extraction



Optimization of Sampling Method

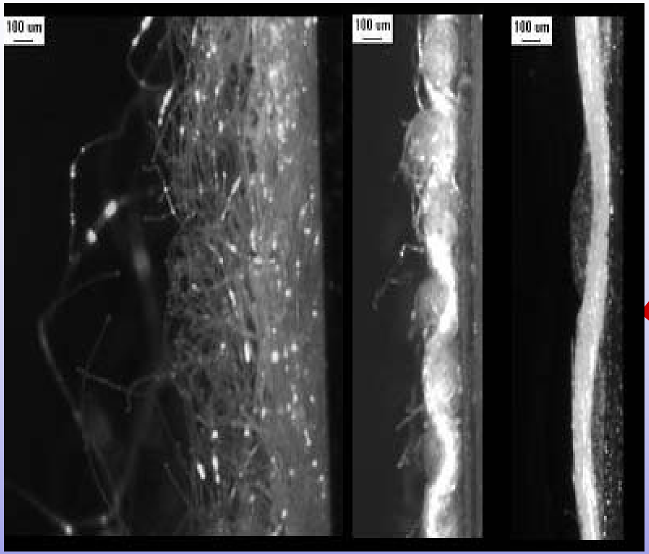
- Environmental Conditions
- Sampling pressure and velocity
- Mass balance on material for loss evaluation
- Wipe and substratum material interactions
- Post-decon impacts on wipe efficiency

Why Develop a Dynamic Wipe Efficiency Method?

- Standard method to evaluate wipe performance in a controlled environment
- Address some of the challenges in surface sampling standards development
- Means for validation of:
  - New materials
  - Integration with new detection technology
  - Address losses and identify gaps in current capabilities
  - Different contaminants and contaminants housed in matrix materials

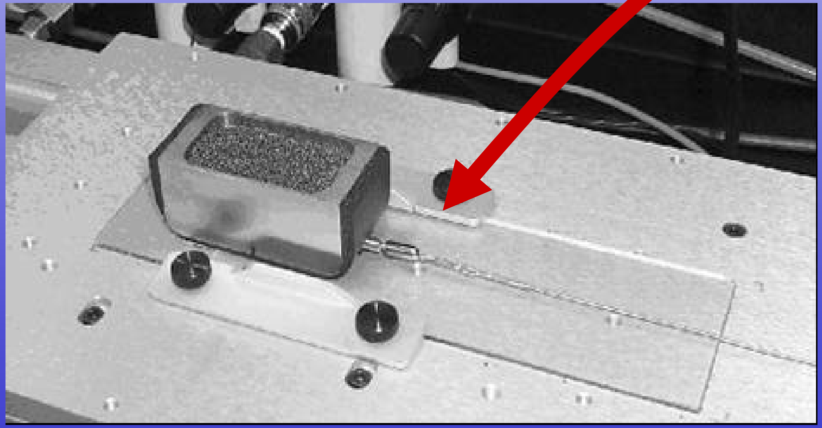
Wipe Materials

Standard Surface Sampling Method to Determine Dynamic Wipe Efficiency

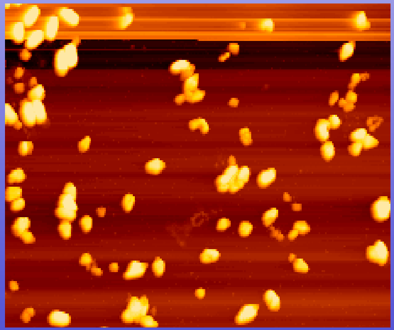


Nonwoven (Swiffer) Muslin PTFE

Controlled sampling procedure to evaluate wipe performance in controlled environment



Crockmeter Testing Device



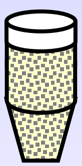
Spores Deposited on Control Surfaces

Modeled on the successful efforts of the
NIST Trace Explosives Group
Metrology of Explosive Particles and Vapors
Sampling, Detection, and Standards

Standard Method for Dynamic Wipe Efficiency

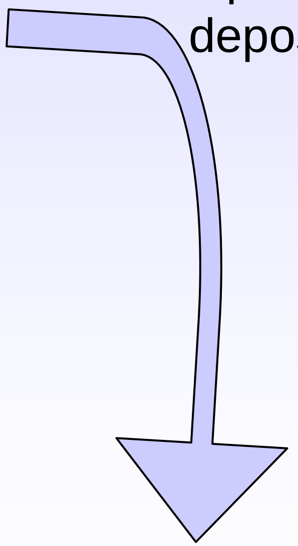
1. Material Characterization

Well characterized aqueous spore suspension



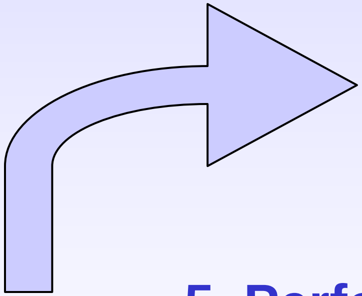
2. Deposition

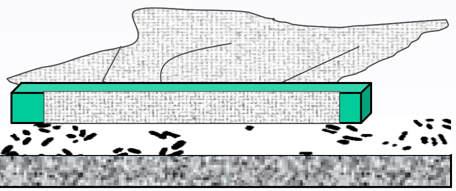
Optically and AFM quantify/characterize deposited spores



3. Wipe Sample

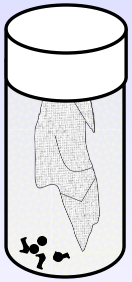
Wipe surface using crockmeter in environmental chamber





4. Extraction

Enhance extraction efficiency with solution chemistry manipulation



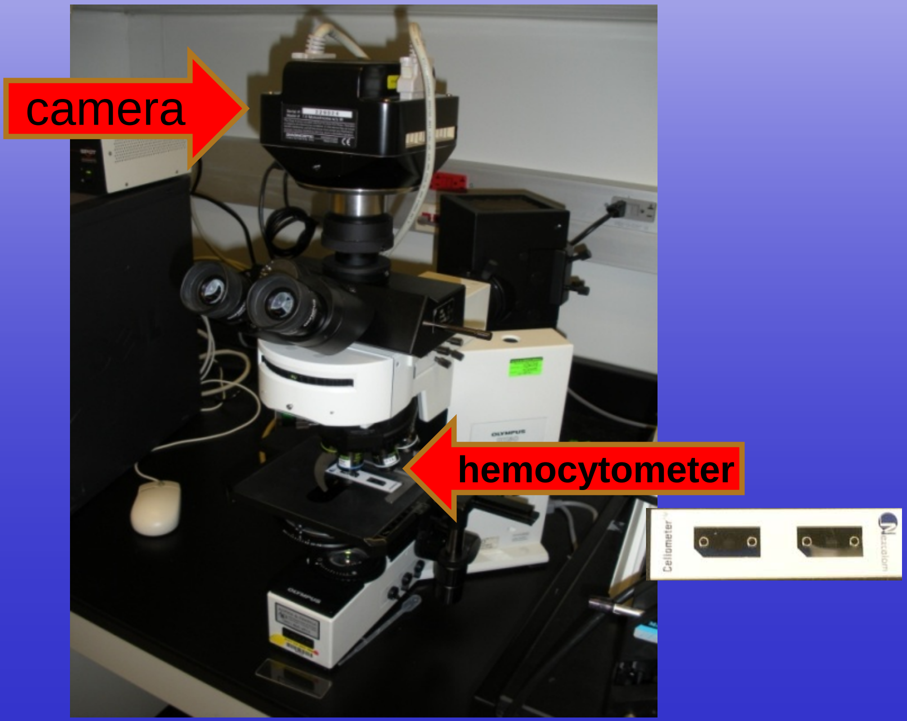
5. Performance

Optically quantify/characterize residual spores

Material Characterization: Spore Quantification

Direct Counts of Preparation Purity

Phase contrast microscopy used to manually count spores on a hemocytometer

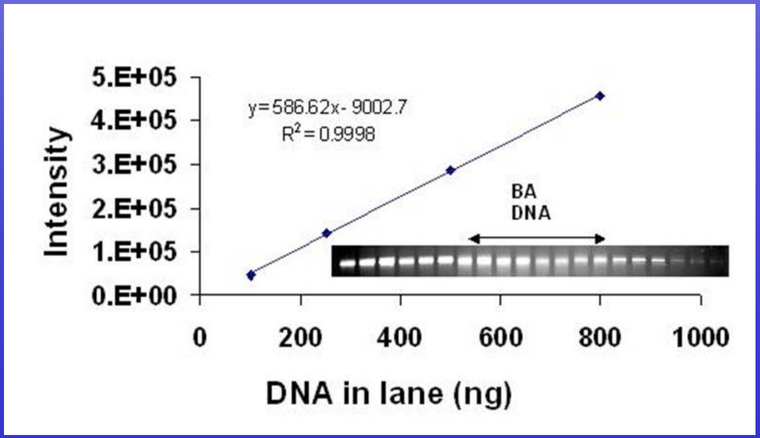


Viability Determination

LB agar plates were spread using serial dilutions and colony forming units (cfu)

DNA Quantity

PCR and gel electrophoresis are used to DNA concentration

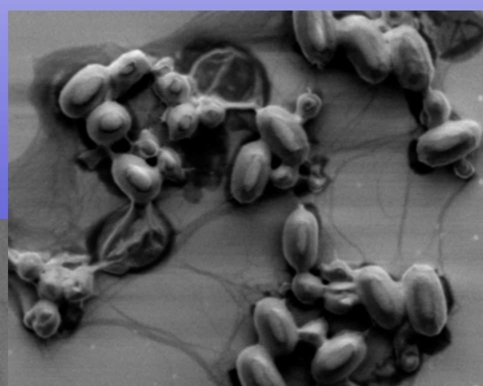


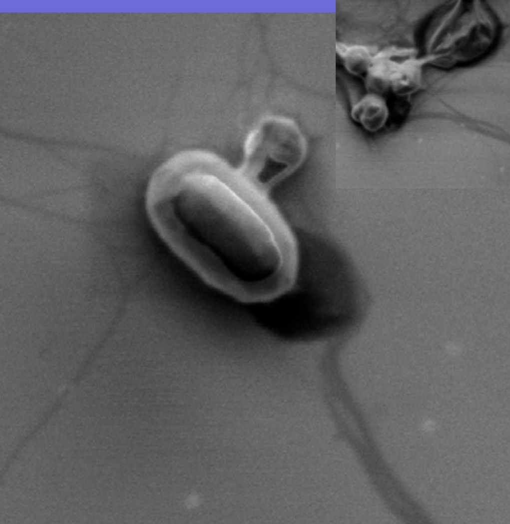
Material Characterization: Spore Aggregation

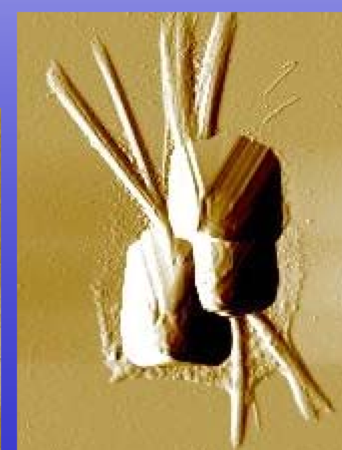
Measurements to aide in reference material production and understanding adhesion of spores to surfaces

- Surface Charge
- Hydrophobicity
- Particle size and morphology

Colloid stability theory to understand aggregation and surface association

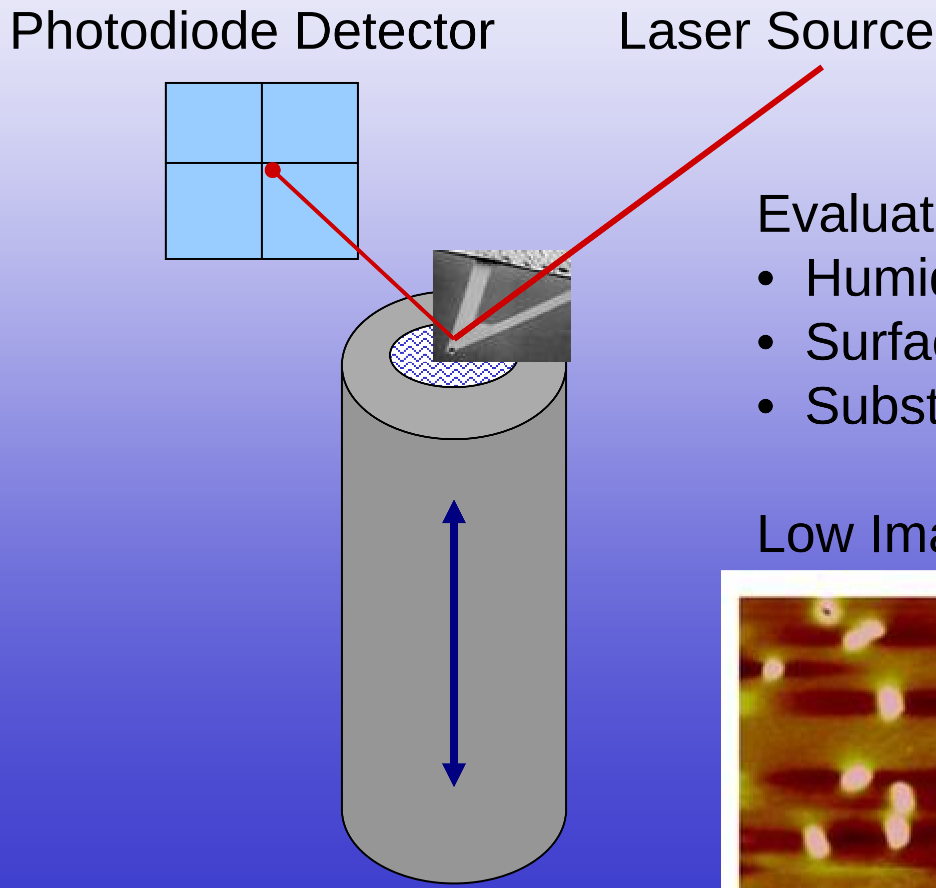






Collaboration with J. Rampersad and D. Ammons from the UTPA and R. D. Holbrook at NIST AML

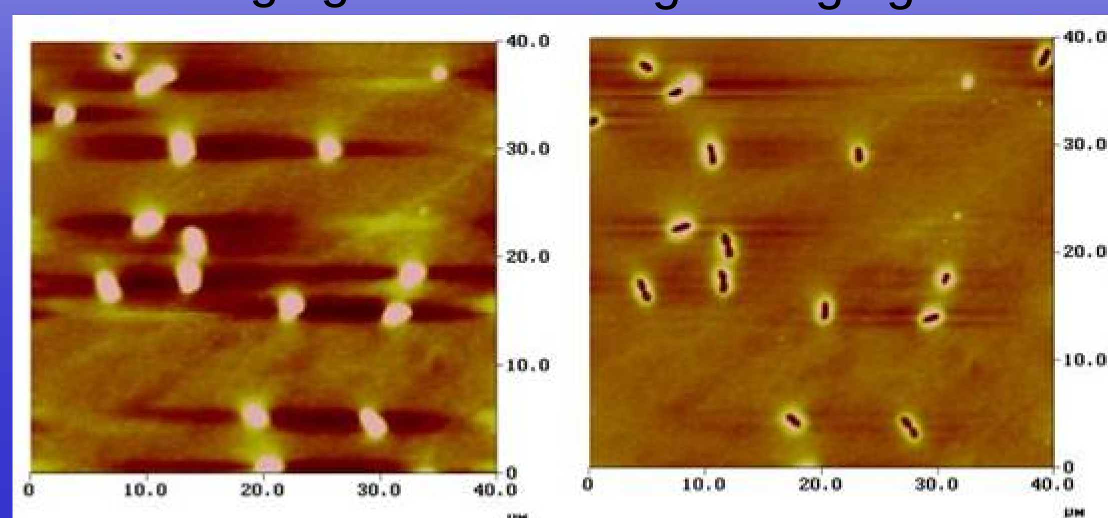
Deposition Technique: Evaluate by Contact Mode AFM



Evaluate Sampling Force Required Given:

- Humidity (RH of 40 - 90%)
- Surfactant (H_2O, volatile Buffer, ± Tween 80)
- Substratum (Glass, SS, Teflon)

Low Imaging Force　　High Imaging Force



Surface Sampling Test Parameters

- Deposition method
- Number of sampling passes
- Substrata: stainless steel, copper, aluminum, glass, Teflon
- Wipes:
  - Woven: cotton, polyester
  - Nonwoven: Versalon (rayon/polyester blend)
- Environmental factors
  - Relative humidity (40 to 90%)
  - Wetting Agent (DI water, PBS, ± Tween 80)
  - Temperature
- Spores embedded in matrix materials
  - Urban particulate dust
  - Arizona dust
  - Skin cells


AVERAGING
STEP
SETUP
STATS

Overview of Efforts at NIST

Measurement Services for Characterized Reference Materials

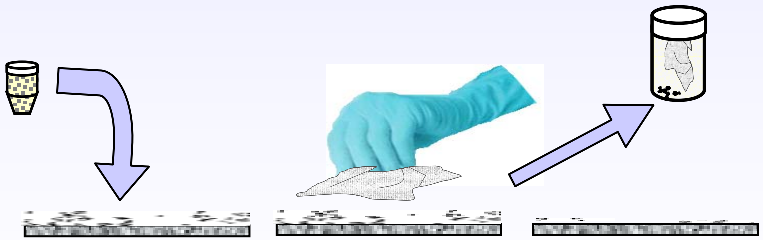
Detection Technology Evaluation and Surface Sampling Science

- DNA Signatures
- Viability Determination
- Optical Characterization
- Surface Chemistry
- Suspension Stability

Guidance document for 1st Responder Technology Selection

Integration of Sampling with Detection Technologies

- DNA Signatures
- Antigenic Signatures
- Viability Determination
- Optical Characterization



Surface Sampling Science

ASTM method for sample collection

ASTM method for wipe efficiency

Guidance document for 1st Responder Surface Sampling

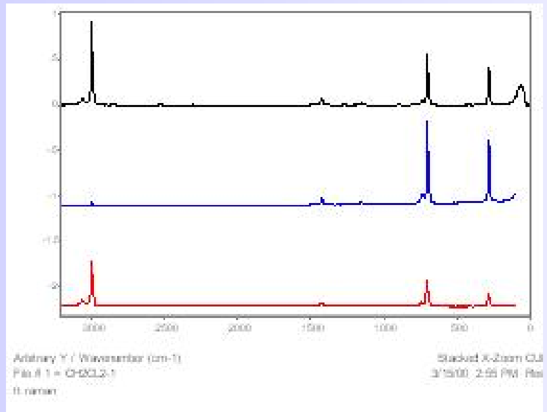
Biological Detectors for First Responders

	Representative Technologies	Basis of the Technology
Screening Devices	Hand Held Assays, Nonspecific particle detection	Antigens, Nucleic acid signatures (**non-PCR**), Proteins Light Scattering/Fluorescence
Field Deployable Detectors	PCR Kit or Integrated PCR Immunological kit	Antigens, Nucleic acid signatures
Autonomous Monitors	Automated PCR, Aerosol detector	Nucleic acid signatures, Spectral properties (fluorescence, Raman)
Laboratory Analysis and Forensics	Standard methodologies (LRN), MIDI, Spectroscopy (FTIR, Raman)	DNA specific (PCR), Fatty Acid, Immunological, Culture Light Spectroscopy

Standards Development for Biothreat Detection by First Responder Community

- Spore reference materials for hand held detection assays
- Spore DNA reference materials for PCR based assays
- Raman spectroscopy reference materials and R&D related to reference material development

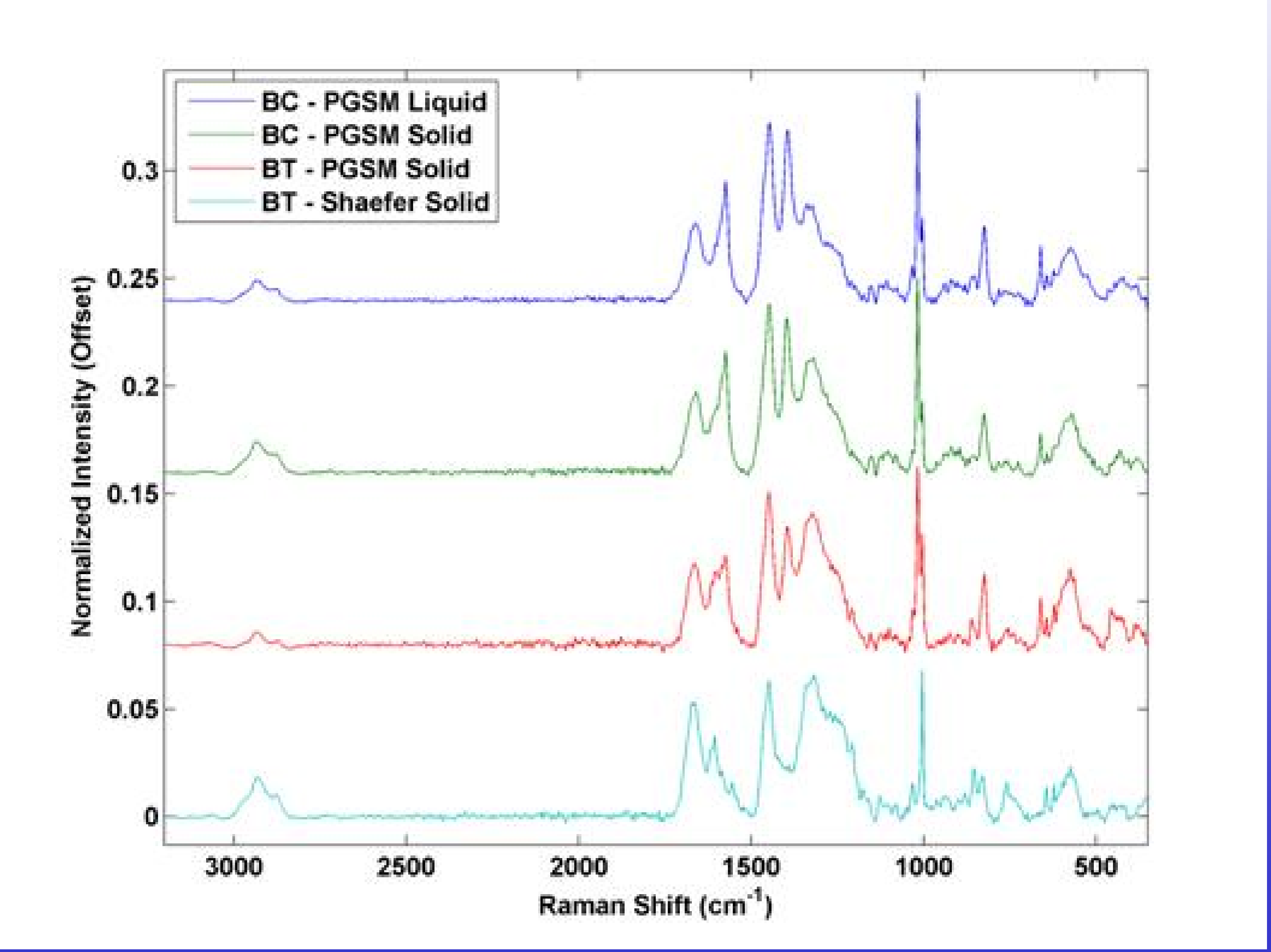
Raman SRM Development



Raman Spectroscopy provides sample identification in real-time without sample preparation and through common containers.

Development of SRM's for calibration of both Raman Shift (x axis), Intensity (y axis) and Resolution.

Raman Spectroscopy- Growth Media Impact and Strain Variation



~60 mW, 785 nm excitation through a 50X objective on our Renishaw system

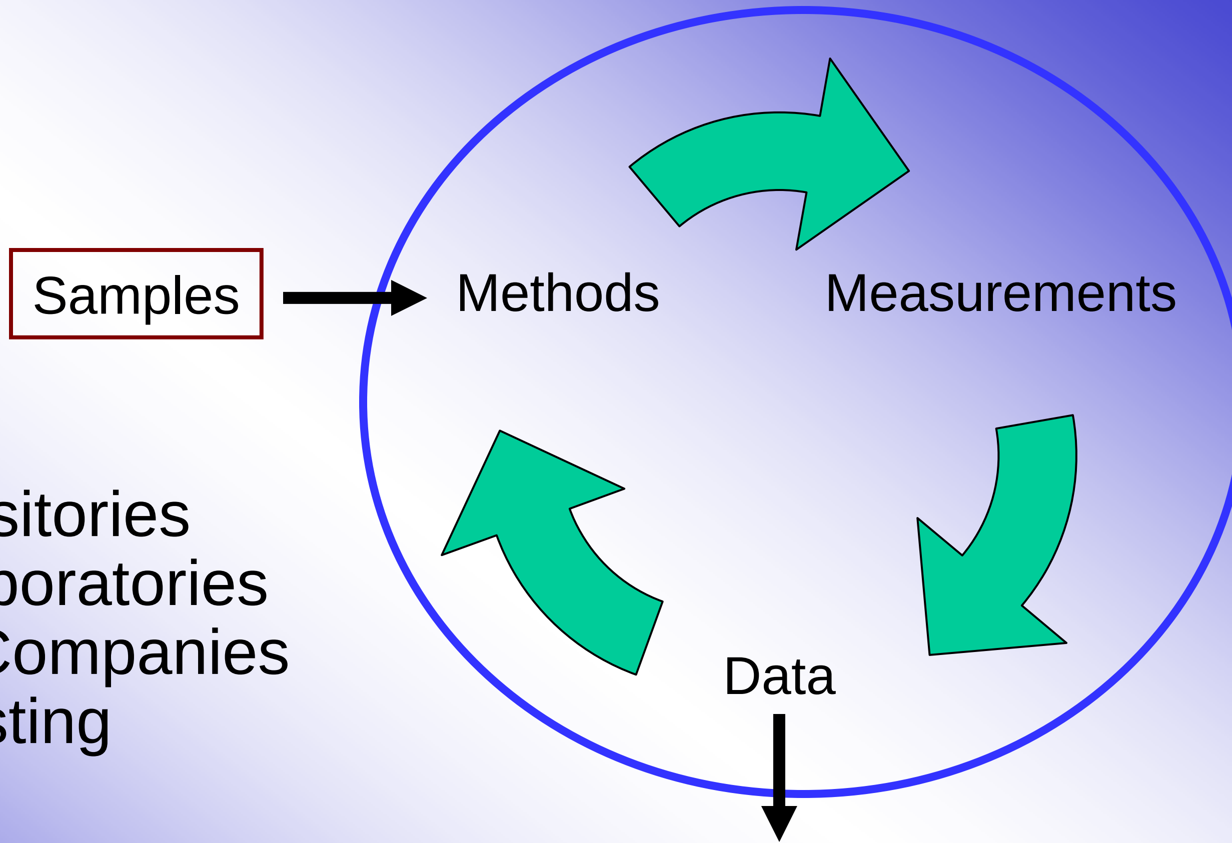
Spore/DNA RM Development

Development of Reference Materials for calibration of detector sensitivity and specificity.

Measurements to support RM development:

- Storage condition impacts on long term stability of spore suspension viability
- Quantification of spore preps
- Spore suspension stability and aggregation factors

Measurement Services



Customers
- Biological Repositories
- Government Laboratories
- Manufacturing Companies
- Commercial Testing Laboratories

Certificates of Analysis to customers (values, uncertainty, methods and recommendations)

Collaborations with USDA and EPA

Future Efforts at NIST

- Improved methods for characterization of reference and training materials
- Measurement characterization of threats and rational selection of simulants and prediction of pathogen fate and transport
- Improved methods for detection or measurement of biological threats and threats in matrix materials including soils, particulate matrices
- Decontamination- data on disinfectants, improved disinfectants and methods for decon
- Sampling and integration of sampling with detection, decon and training on sampling methods

ACS Environmental Division Call for Papers

We would like to draw your attention to an upcoming symposium for the 238th American Chemical Society National Meeting in Washington DC from August, 16-20, 2009. This symposium within the Division of Environmental Chemistry is titled **“Detection and Sampling for Biodefense”.** The official Call for Papers can be found at **http://www.environfacs.org.**

The purpose of this session is to foster innovative interdisciplinary approaches to studying the integration of biological detection and sampling technologies by attracting researchers with a variety of scientific backgrounds who are studying this and related topics. This session will present recent advances in (i) innovative quantitative or mechanistic detection technologies, (ii) measurement issues in the integration of detection technology with surface and aerosol sampling methodologies (ii) bioanalysis in complex and environmental matrices and (iii) surface chemistry contributions to detection and sampling efficiency. We are particularly interested in work that evaluates the challenges in detection of specific microbial pathogens and biotoxin analytes relative to the biodefense community. Presenters are required to submit a short abstract to the ACS by March 16, 2009, using the ACS on-line system (OASYS) at http://oasys.acs.org/. **OASyS opens on January 19, 2009 and closes March 16, 2009.** There is no requirement for an extended abstract.

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